Naval Health Research Center Detachment (Toxicology)

PULMONARY FUNCTION IN NORMAL RATS

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PREFACE - EXECUTIVE SUMMARY

This research was conducted at the Naval Health Research Center Detachment (Toxicology) and was sponsored by the Naval Medical Research and Development Command under Work Unit # 63706N-M00095.004.1714 entitled "Investigation of the mechanisms and Pathogenesis of the Acute Respiratory Distress Syndrome (ARDS) Related to Smoke Inhalation." The objectives of this work unit are to develop a better understanding of the severely debilitating and often fatal lung diseases Acute Lung Injury and Acute Respiratory Distress Syndrome (ALI/ARDS). The principal focus of the research is an examination of the role that smoke inhalation plays in the development of ALI/ARDS. Inhalation of smoke and combustion atmospheres from shipboard and other fires represents a significant health risk to Naval personnel. To date, there is not a well-developed treatment regimen for ALI/ARDS and prevention programs are not adequate to protect all personnel at risk. There are no techniques for early warning of risk either biologically or from the perspective of environmental monitoring. Furthermore, the role that many of the most common constituents of smoke and combustion atmospheres play in the development of this family of lung diseases is not clear. There are, as yet, no biomedical indicators of susceptibility to or for the final outcome of ALI/ARDS, which can be exploited to protect personnel. Identification of the mechanisms of development of the disease and the agents in smoke and combustion atmospheres which are most responsible for induction of ALI/ARDS is needed for the development of successful medical intervention strategies and the development of early warning devices which may minimize risk. For a more detailed treatment of ALI/ARDS, militarily relevant inhalation injury, and risk from combustion atmospheres refer to Kimmel and Still, 1999.

The development of models of ALI/ARDS is needed evaluate risk associated with the inhalation of combustion atmospheres of new and exotic materials being placed into use in Naval systems. These models are necessary for the assessment of potential treatment regimens. Identification of principle causative agents in smoke and development of predictive models of their role in ALI/ARDS also represents an important aspect of smoke health effects research.

The purpose of the development of the pulmonary function tests (pfts) presented here in "Pulmonary Function in Normal Rats" is to use them as physiological biomarkers of underlying pulmonary disease. Inhalation exposure to toxins often leads to changes in pulmonary physiology (hence pulmonary function) in ways that are diagnostic of the type and severity of the disease. In humans ALI/ARDS causes many characteristic physiological changes in lung function. Some of which are pathognomonic of the disease. In most cases human pfts have correlates in small animals and corresponding changes in these small animal pfts reflect anatomical, biochemical and physiological changes which are similar to those that occur in human ALI/ARDS. Thus pfts are an important tool for the assessment of ALI/ARDS in small animal models of the disease. However in small animals, it is often necessary to collectively analyze a battery of various pfts in order to gain a clear diagnostic picture. Thus large batteries of tests are required and usually performed where in humans other diagnostic measures may be used.

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research, National Research Council, DHHS, National Institutes of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended.

The opinions herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval.

TABLE OF CONTENTS

ABSTRACT

Pulmonary function tests (pfts) often are used in both humans and small animal species as physiologic biomarkers of pulmonary disease caused by the inhalation of toxic atmospheres. Physiologic biomarkers, used in conjunction with histopathological and biochemical biomarkers, can be used to diagnose disease, characterize dose/response relationships, assess disease pathogenesis, and indicate the degree of debilitation following pulmonary insult. We have developed methods to perform a battery of pfts that will be used to assess acute and chronic pulmonary toxicity in small animals exposed to inhaled toxins. The pfts developed can be used to assess physiologic responses in real-time during exposure, post exposure progress of induced pulmonary lesions, or both. Included are measures of ventilation (frequency, tidal volume, and minute ventilation), breath waveform analysis (flow derived parameters), dynamic pulmonary mechanics (compliance and resistance), static pulmonary mechanics (lung pressure-volume relationships and quasistatic compliance), sub-divisions of lung volume, pulmonary dynamics (forced maneuvers), distribution of ventilation (single breath N_2 washout), gas exchange (carbon monoxide - single breath diffusing capacity, microcapnometry), and measurement of metabolic activity (microcapnometry). Sixty one untreated Long Evans rats were used to develop the assays and to form a historical database for normal animals. Values for a variety of indices of pulmonary function measured using the methods developed in our laboratory were comparable to those reported by several other investigators.

KEY WORDS

Pulmonary, ventilation, lung compliance and resistance, pulmonary mechanics, gas exchange, and metabolic rate.

DISCLAIMER

The work reported herein was conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research, National Research Council, DHHS, National Institutes of Health. Publication 85-23, 1985 and the Animal Welfare Act of 1966, as amended.

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LIST OF ABBREVIATIONS

Note: common chemical and measurement abbreviations are not included.

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
pfts	Pulmonary Function Tests
f	Frequency
MV	Minute Ventilation
Vt	Tidal Volume
C_{dyn}	Dynamic Compliance
R_L	Resistance
PV	Pressure-Volume
TLC	Total Lung Capacity
ERV	Expiratory Reserve Volume
VC	Vital Capacity
FRC	Functional Residual Capacity
Ti	Inspiratory Time
Te	Expiratory Time
RT	Relaxation Time
EIP	End Inspiratory Pause
PENH	Enhanced Pause
D_{LCO}	Diffusing Capacity for Carbon Monoxide
Ptt	Trans-thoracic Pressure
Pao	Pressure at Airway Opening
IC	Inspiratory Capacity
Cqst	Quasistatic compliance
RV	Residual Volume
FVC	Forced Vital Capacity
FEV	Forced Expired Volume
EF	Expiratory Flow
EF_{50}	Expiratory Flow at 50%
EF_{25}	Expiratory Flow at 25%
EF_{10}	Expiratory Flow at 10%
MMEF	Mean Midexpiratory Flow
IES	Initial-Expiratory Sample
EES	End-Expiratory Sample
Vr	Volume of Relaxation

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Measures of pulmonary function are used to evaluate lung response to inhaled toxins. Pulmonary function tests (pfts) are commonly used for diagnosis of the nature and severity of lung injury. Physiologic markers offer the advantage of being relatively non-invasive compared to biochemical and pathologic biomarkers of lung injury and disease. However, pulmonary function tests are not as sensitive to specific pathologies as are biochemical markers. They often are less informative about the mechanisms of injury and disease than are biochemical and pathologic markers. Consequently, numerous pfts have been developed to examine specific aspects of lung and respiratory function. Through collective evaluation of several pfts one can distinguish basic categories of damage underlying the pathology and occasionally distinct lesions (Costa et al., 1986 and Mauderly 1988).

Among the various types of pfts are those that are well suited for assessment of acute respiratory responses to toxic exposure. These acute responses are generally rapid in onset, transient, and are not necessarily associated with underlying tissue damage. An example of this type of response is airway hyperresponsiveness. Physiologic measures of ventilation, breathing frequency (*f*) and tidal volume (Vt), are often used as indices of acute response to inhaled toxins (Karol et al., 1985). So-called flow-derived parameters that examine breath structure such as inspiratory time (Ti), expiratory time (Te), relaxation time (RT), end inspiratory pause (EIP) and enhanced pause (PENH) have also been used as indices of acute response to inhaled toxins (Comroe 1975). These relationships are illustrated in Figure 1. Classic indicators of the mechanical behavior of the respiratory system are also often used to evaluate acute response to inhaled toxins (Amdur and Mead, 1958). The mechanical behavior of the lungs includes its elastic, resistive, and inertial forces. Dynamic properties of the mechanics of breathing include lung dynamic compliance (C_{dyn}) and resistance (R_L) and measures of flow-volume curves. Static measures are often used to examine the elastic properties of the lung. Inflation and deflation pressure-volume (PV) curves are used for this purpose (O'Neil and Raub 1984). Total lung capacity (TLC) is generally defined at a given transpulmonary pressure. PV curves are measured over volume range from expiratory reserve volume (ERV) to TLC, also known as vital capacity

(VC). These volumes coupled with the measurement of functional residual capacity (FRC) can be used for an analysis of the subdivisions of lung volume as indices of pulmonary dysfunction (DuBois et al., 1956). Measures of the distribution of ventilation (single breath N_2 washout) are often used as indicators of small airway disease.

Coupled with analyses of breathing and pulmonary mechanics one can examine lung gas exchange properties using two techniques. Alveolar-capillary membrane integrity can be assessed by determining the rate of gas transport across that membrane. Diffusing capacity for carbon monoxide (D_{LCO}) is used for this purpose because of relatively high rate for diffusion of CO across the membrane (Takezawa et al., 1980). Microcapnometric measurements of CO_2 concentration at the beginning and end of a breath also can be used as an indicator of lung gas exchange properties and basal metabolic rate (Murphy et al., 1994).

We have developed techniques to measure a variety of pfts in small animals for use in toxicity studies at the Naval Health Research Center. These pfts employ barometric, volume displacement, and constant volume plethysmographic techniques. We wished to compare our findings for a variety of individual pfts, in untreated rats, to those reported in the literature. We used two variants of volume displacement plethysmography to make our measurements. One is a well-known technique in which the animal is fully enclosed in the plethysmograph, called a Diamond box after L. Diamond (Diamond and O'Donnell, 1977, Kimmel and Diamond, 1984). The second is a recently developed method that combines head-out techniques with the use of an esophageal cannula to measure intrapleural pressure. For our purposes the latter method is referred to as head-out plethysmography. We used a combination of these techniques to measure sub-divisions of lung volume, lung pressure-volume relationships and quasistatic compliance, pulmonary dynamics (forced maneuvers), distribution of ventilation (single breath N_2 washout), and gas exchange (carbon monoxide - single breath diffusing capacity). Each of these plethysmographic techniques offers distinct advantages for measurement of these pfts under a variety of circumstances. Consequently some values for individual pfts may be reported separately for each technique. We also developed microcapnometric techniques to measure gas exchange function and metabolic rate.

METHODS

ANIMALS

Sixty-one female Long Evans rats (220-290g) used in this study were obtained commercially (Charles River Laboratories, Raleigh, NC). Prior to initiation of the investigation two animals were selected at random, sacrificed and examined by a veterinary pathologist. The animals were found to be in good health. Until use the animals were housed in plastic shoebox type cages suspended over adsorbent bedding, and were maintained on a 12 hr. diurnal cycle. Food and water were provided *ad libitum*. Prior to barometric and flow box techniques the animals were lightly anesthetized with urethane (ethyl carbamate, i.p. - 0.75 g/kg). Prior to use of the Diamond box and microcapnometry the animals were anesthetized to surgical depth and a tracheal cannula (3 way connecting tube, 0.24 cm i.d., Small Parts Inc., Miami Lakes, Fla.) was inserted. For PV curve measurement and determination of D_{LCO}, the Diamond box was configured as a constant volume plethysmograph by plugging the pneumotachograph. Both of these pfts require measurement of trans-thoracic pressure (Ptt). Ptt is the difference of ambient pressure and pressure at the airway opening (Pao) during these maneuvers. Ambient pressure in this instance is the pressure within the plethysmograph. Pao is measured by connection of the pressure transducer to the sidearm of the tracheal cannula. Prior to measurements of D_{LCO} the animals were paralyzed with gallamine triethiodide (i.v. – 2.0 mg/kg). Following pfts, the animals were euthanized with sodium pentobarbital overdose (i.v. - 60 mg/kg).

BAROMETRIC PLETHYSMOGRAPHY TECHNIQUES

Respiratory flows and volumes were measured in nine rats using modified barometric techniques modified from those described by Drorbaugh and Fenn, 1955. A 4.5 - L whole-body plethysmograph for use of unrestrained animals and with built in pneumotachographs (Model 3215, Buxco Electronics, Sharon, CT) was used. This technique uses a 750 ml/min regulated bias flow through the plethysmograph (model PLY/BF-M, Buxco Electronics, Sharon, CT). A differential pressure transducer (Model S0X1, 0 - 1 psi, SenSym Inc., Milpitas, CA) was used to measure the pressure differential across the pneumotachograph to determine flow. The pressure

differential between the inside of the box and a reference space is used. The flow through the pneumotachograph was linear to the differential pressure and was integrated. Transducer signals were conditioned using an amplifier (Model No. PREAMP/Val (6), Buxco Electronics, Sharon, CT), digitized, and processed in real time (Model No. DL-12/24, Buxco Electronics, Sharon, CT). Real time calculations of Vt, *f* and flow derived parameters were performed and recorded electronically by computer software (BioSystem XA software, Buxco Electronics, Sharon, CT).

VOLUME DISPLACEMENT PLETHYSMOGRAPHY

HEAD-OUT TECHNIQUES

A head-out plethysmograph was used for measurements of respiratory flows, waveform analysis and lung mechanics. A pneumotachograph consisting of 6 layers of #325 wire mesh screen located across a circular 1.0-cm.-diameter port was used to measure flow. Flow in and out of the plethysmograph was proportional to the pressure differential across the pneumotachograph. A differential pressure transducer (Model DP 45-14, Validyne Engineering, Northridge, CA) was fitted into the side-wall of the plethysmograph positioned opposite of the pneumotachograph. A tracheal cannula was not used.

Real time measurements of Vt, *f*, and flow-derived parameters were performed on 12 rats and recorded. Simultaneously, intrapleural pressure was measured so that C_{dyn} and R_L could be calculated (Amdur and Mead, 1958). Intrapleural pressure was measured by an esophageal cannula. The water-filled cannula, free of any air bubbles, was transorally inserted into the esophagus. The cannula was connected via a three way stop cock to a water filled pressure transducer which also was free of air bubbles (Model No. SX01DN, 0 to 1 psi, sense Inc., Milpitas, CA). All data were recorded by Buxco software described earlier.

DIAMOND BOX TECHNIQUES

Respiratory flows, waveform analysis, and lung mechanics were measured in 30 rats using a Diamond box (Model PLY3114, Buxco Electronics, Sharon, CT). Diamond box techniques differ from head-out techniques in that it uses a tracheal cannula. A control unit (Max II Maneuver Signal Generator, Buxco Electronics, Sharon, CT) was used to control valves and condition signals. A differential pressure transducer (Model S0X1, 0 - 1 psi, SenSym Inc., Milpitas, CA) was used to measure the pressure differential across a pneumotachograph to measure flow. A pressure transducer (Model TRD4510, Buxco Electronics, Sharon, CT) measured pulmonary pressure. Intrapleural pressure was measured by a water-filled transducer (Model TRD0113, Buxco Electronics, Sharon, CT) connected to a water-filled esophageal cannula that was transorally inserted into the esophagus. The sidearm of the tracheal cannula was plugged for these procedures. Real time calculations of Vt, *f*, flow derived parameters and C_{dyn} and R_L were performed and recorded electronically to a file by computer software (Biosystems XA software, Buxco Electronics, Sharon, CT).

Single breath N_2 washout curves, PV curves, quasistatic compliance, and pulmonary dynamics were measured using the Diamond box configured as a volume displacement plethysmograph. We used the same 30 rats for each procedure.

Single breath nitrogen washout curves were measured using the same techniques as Mauderly, 1984. The animal's lungs were inflated to TLC with 100% oxygen and N_2 concentration was measured continuously in the expired airflow. N_2 concentration was plotted versus time/volume resulting in the typical N_2 washout curve shown in Figure 3. A nitrogen analyzer (model 47302 A, Hewlett Packard Inc., Waltham, MA) measured nitrogen concentration at the airway opening. Different phases of the N_2 washout curve coincides with N_2 gas being washed out of different parts of the respiratory tree. The slope of phase III was how evenly the alveolar space was being emptied. Information regarding physiologic dead space and closing volume was derived from these curves.

PV curves were measured by methods similar to those of Kimmel and Diamond, 1984. With the Diamond box in the constant volume configuration, volume was determined as the pressure within the box. The pressure component of the curve corresponds to Ptt, which was the difference between the pressure within the box and Pao. PV curves were generated by plotting volume versus pressure over the course of controlled inflation of the lung. This was done from FRC to TLC. The difference between TLC and FRC was defined as inspiratory capacity (IC). See Figure 4. The slope of the steepest portion of the expiration leg was used to determine quasistatic compliance (Cqst). TLC was the total volume of the lung at maximum inflation defined at a Ptt of 30 - cm H_2O. Likewise, ERV was the maximum volume that can be withdrawn by controlled deflation from Ptt = 0 cm H_2O. VC = IC + ERV. Residual volume (RV) was defined as the volume remaining after maximal deflation and equals FRC-ERV.

FRC was determined in spontaneously breathing animals with the Diamond box configured as a constant volume plethysmograph. FRC was defined as the lung volume remaining in the lung at end expiration. FRC was determined by applying Boyle's law to volume/pressure changes during breathing efforts against an occluded airway (DuBois et al., 1956).

Expiratory flow-volume curves were measured in 30 animals using similar techniques described by Diamond and O'Donnell, 1977. The lungs were inflated to TLC, and then the airway opening was connected to a reservoir maintained at - 50.0 cm H_20. A typical flow-volume curve is shown in Figure 5. We measured forced vital capacity (FVC). The vital capacity represents the range of lung volume that an animal can voluntarily breath. We measured the forced expired volume (FEV) at 0.1, 0.2 and 0.3 seconds. In addition, we measured expiratory flow at 50, 25, 10% of FVC (EF_{50}, EF_{25}, and EF_{10}, respectively). Mean midexpiratory flow (MMEF) was also measured.

D_{LCO} was measured in these 30 rats by the method of Sabo et al., 1984. Lung volume as a function of time was plotted on an XY recorder (Model LY 1900PL, Lineis Inc., Princeton, N.J.). An amount of CO/Ne (approximately 0.5% each) test gas mixture equal to the animal's IC was injected into the animal's lungs and held for ten seconds. The gas was extracted in two parts,

initial-expiratory sample (IES) and the end-expiratory sample (EES). The IES was considered as dead space, therefore CO concentration in the EES was used to determine D_{LCO}. Ne concentration in both the IES and EES were used to determine volume of relaxation (Vr), a gas dilution analog of FRC (not shown). A typical D_{LCO} plot is shown in Figure 6. The concentrations of CO and Ne in the IES and the EES were then analyzed by a gas chromatograph (model GC-8A, Shimazdu Inc., Kyoto, Japan).

MICROCAPNOMETRY

Changes in CO_2 concentration measured at the airway opening during a course of a breath was measured using microcapnometic techniques described by Murphy et al., 1994. End-tidal CO_2 was measured for each breath in 8 spontaneously breathing rats with a microcapnometer (Model 0151-004L, Columbus Instruments Inc., Columbus, OH). A 20-ml probe sampled inspired and expired air. The tip of the sampling probe was positioned in the airstream by placing it inside the side arm of the cannula, which was sealed with para-film.

STATISTICS

Pfts measured in spontaneously breathing animals were recorded for 10-min. intervals. Typically a minimum of 500 breaths were analyzed to calculate an average value for that animal. The values that are reported are the mean of these animal averages.

Volume-pressure curves, expiratory flow-volume curves, single breath N_2 washout tests, D_{LCO}, and subdivisions of lung volume were determined three times for each animal. The mean values for these determinations were recorded for each animal. The values that are reported are the means of all animals undergoing evaluation.

Microcapnometry data were measured in each animal for a 10-min. period. Mean values for each animal were derived from these data.

RESULTS AND DISCUSSION

Measures of ventilation and flow derived parameters obtained from normal rats using barometric, head-out and Diamond box plethysmography are shown in Table 1. Measures of respiratory mechanics, C_{dyn} and R_L, obtained using head-out and Diamond box plethysmography also are shown in Table 1. One should exercise caution when making comparisons of ventilation and flow-derived parameters between barometric and volumetric plethysmography, of either type. The accuracy of barometric methods has been questioned, particularly with the regard to volume measurements (Costa 1985). Likewise, care should also be taken when comparing C_{dyn} and R_L between the two techniques of volume displacement plethysmography used. The Diamond box technique employs a tracheal cannula thereby bypassing the nasal vestibule. Despite differences in techniques, all values of ventilation and flow derived parameters, except PENH and EIP, were of the same magnitude for all three plethysmographic techniques. The values of PENH and EIP values were significantly greater when the Diamond box was used. This may have been due to the use of a tracheal cannula, and a presence of a valve assembly on the breathing port. The slight, but insignificant, differences of C_{dyn} and R_L may also have been due to the same reasons. Consistent use of one technique in a toxicity study eliminates concern regarding subtle methodological differences in measurement in a given parameter.

TV, *f*, MV, R_L and C_{dyn} obtained in our laboratory were compared to values published in the literature. See Table 2. The mean and standard deviations for each observation in the literature were only given for comparison. We found a wide range of published values for these variables even though our search was not exhaustive. We attempted to compare our data to published data collected using comparable fundamental methods. One of the largest discrepancies found was a slightly greater than two-fold difference between our C_{dyn} value and that of Diamond. Nether the less, our values fit well within the range of published values.

Values of sub-divisions of lung volume obtained from 30 rats using the Diamond box are shown in Table 3. These values were collected from a variety of maneuvers: FRC determinations, PV curves, forced expiratory flow-volume curves, and D_{LCO}. Using methods

similar to ours, Harkema et al., 1982, reported lung volume data for male and female F344 rats. The largest discrepancy found was for RV/TLC, where the ratio of our value to Harkema's was 18 to 10. However, the values are comparable when the raw data are normalized for body weight differences.

For our microcapnometry techniques, we found a similarity between our results and those of Murphy et al., 1993. In our laboratory eight spontaneously breathing rats (635 ml/kg-min) had an end-tidal CO_2 value of 5.15 ± 0.2 %. The average CO_2 concentration at inspiration was 0.4 % CO_2. Murphy found end-tidal CO_2 values ranging from 4-6 % in animals ventilated mechanically at 750 ml/kg-min.

We are satisfied that are methods are suitable for use in toxicity studies. For the most part, our data compare well with those of numerous investigators that use pulmonary function testing to evaluate lung response to inhaled toxins (Mauderly, 1988 and Costa and Tepper, 1988). We have developed three plethysmographic techniques, each with specific applications to toxicology studies. Barometric plethysmography, although indirect and subject to some error, is a convenient, non-invasive method to examine airway reactive responsiveness and airway hypersensitivity. Head-out plethysmography is suitable for measurement of ventilation and respiratory mechanics during exposures. The Diamond box, configured either as a volume displacement or a constant volume device, can be used for a wide variety of pulmonary function tests gathered post exposures. All three of these can give a comprehensive view of the lungs response to an inhaled toxin.

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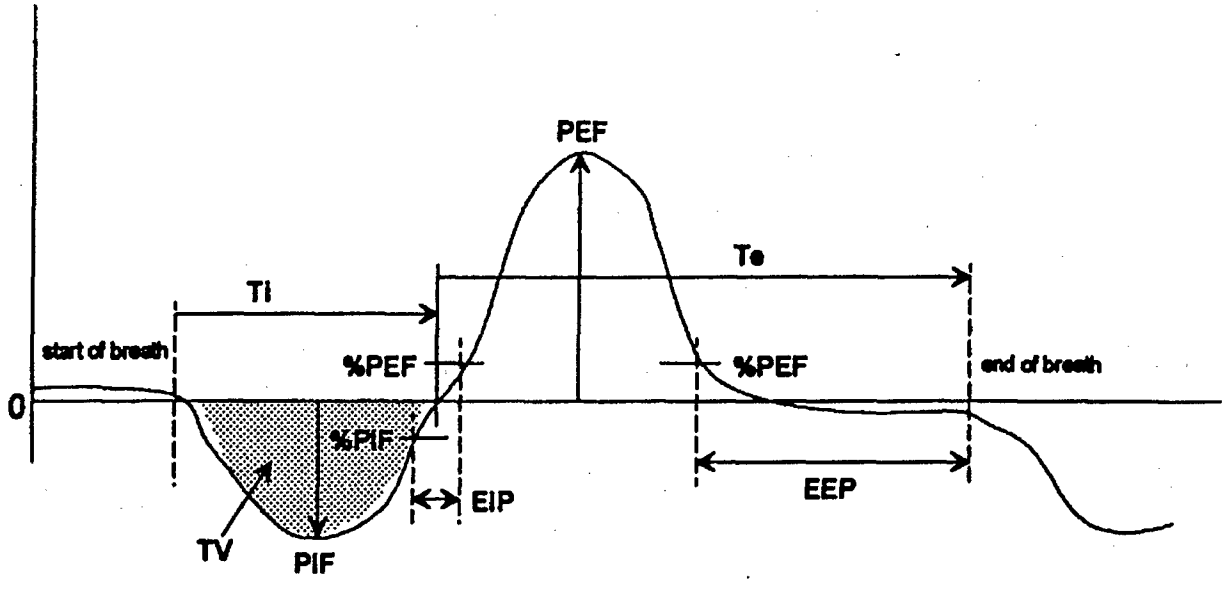
Figure 1. Schematic diagram of a tidal breath - flow derived parameters from Buxco Electronics, Inc. - with permission. Expiration - above 0 flow axis (x-axis)



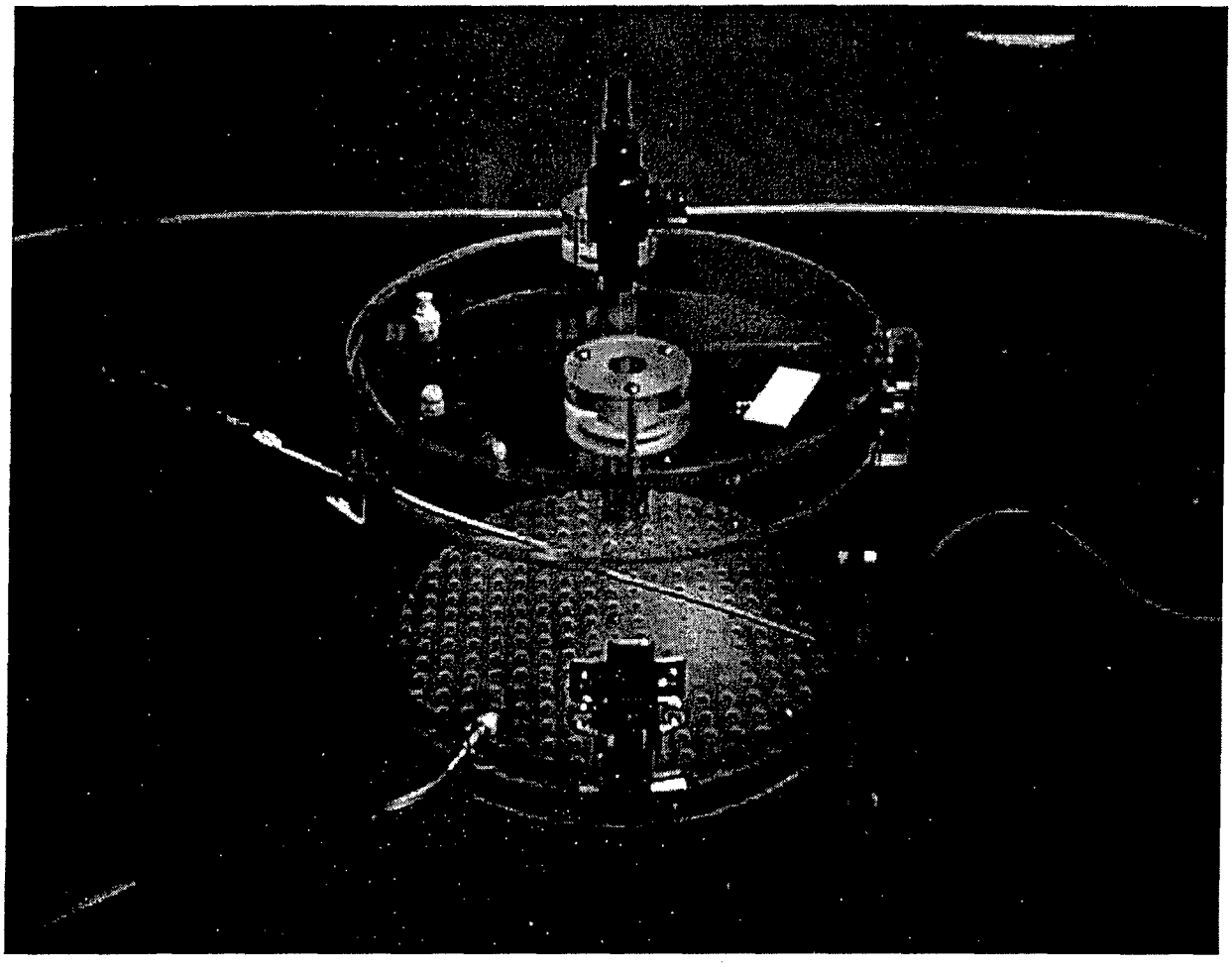
Abbreviations and Units:

Ti	Inspiratory Time (time of start of inspiration to the end of inspiration in seconds)
Te	Expiratory Time (time of start of expiration to the end of expiration in seconds)
TV	Tidal Volume, the volume of each breath (ml)
f	Respiratory Frequency (breathes per minute)
MV	Minute Ventilation (Tidal volume * frequency/min)
RT	Relaxation Time (seconds)
PIF	Peak Inspiratory Flow (ml/s)
PEF	Peak Expiratory Flow (ml/s)
EIP	End Inspiratory Pause (seconds)
EEP	End Expiratory Pause (seconds)
PENH	Enhanced Pause [(Te/RT-1)(PEF/PIF)]

Abbreviations not found on diagram

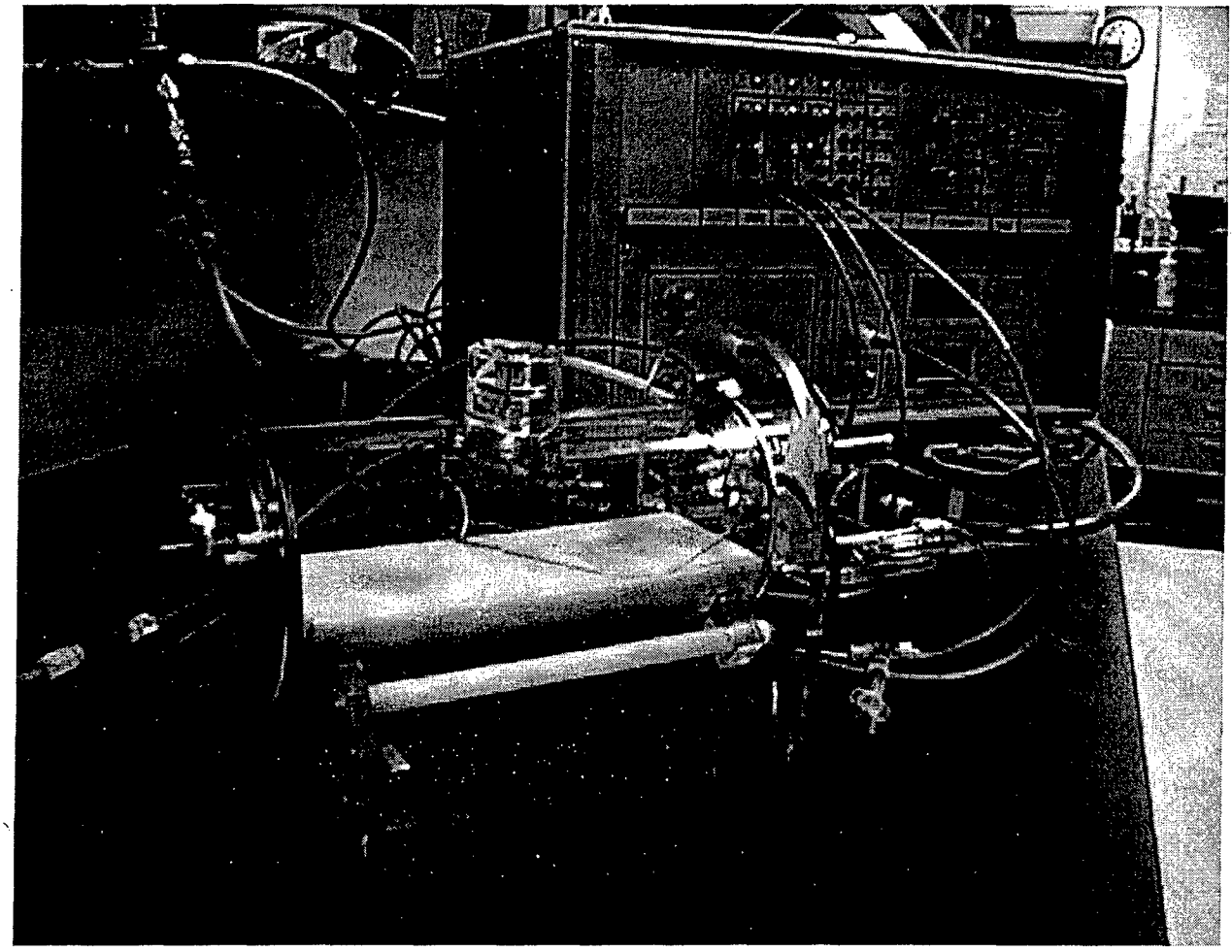
R_L	Pulmonary resistance (dP/DF The change in pressure divided by the change in flow)
C_{dyn}	Dynamic Lung Compliance (dV/dP The change in volume divided by the change in pressure)

Figure 2. Barometric Plethysmograph



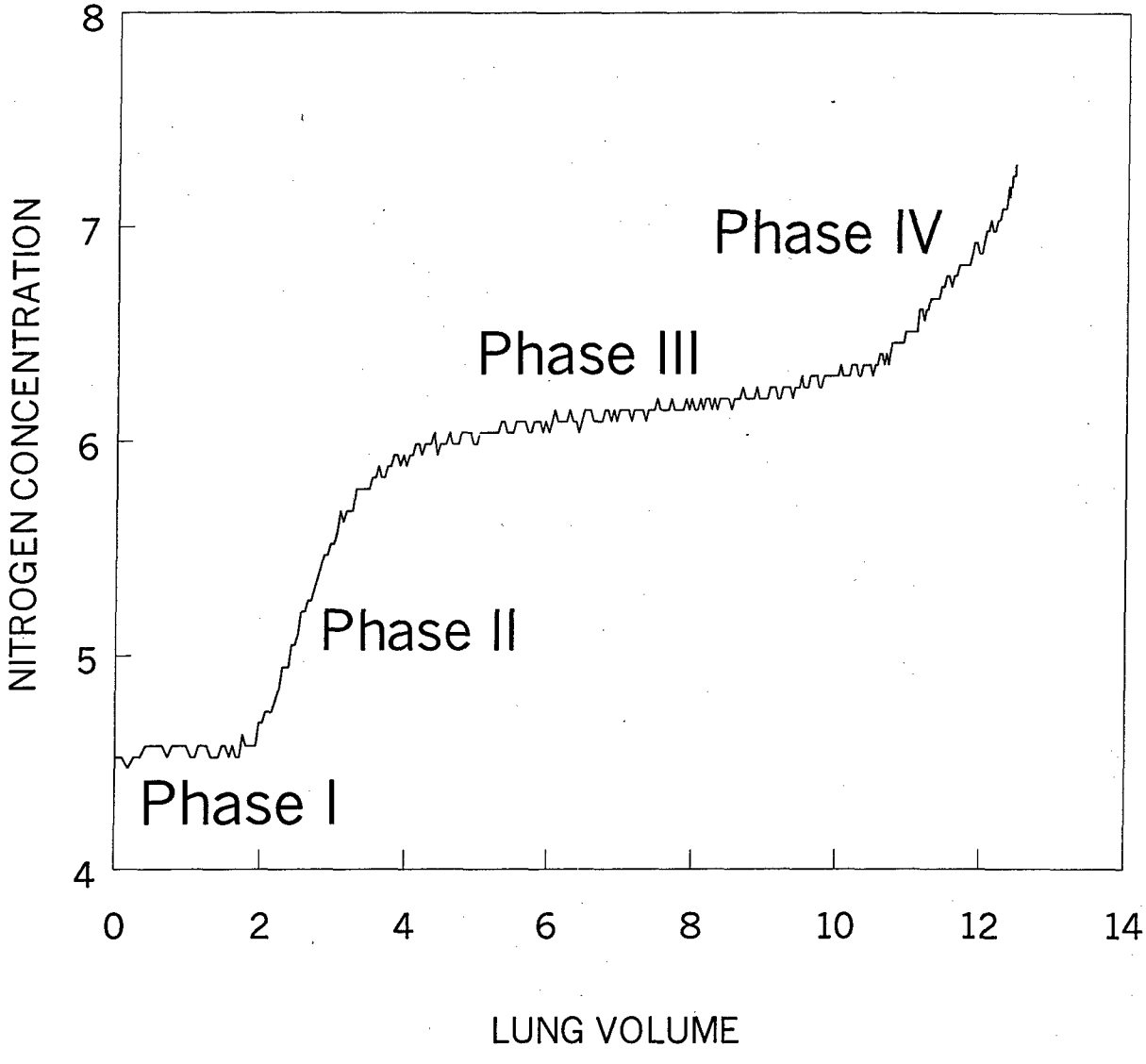
A picture of a 4.5 L whole-body plethysmograph (model 3215, Buxco Electronics, Sharon, CT).

Figure 3. Diamond Box



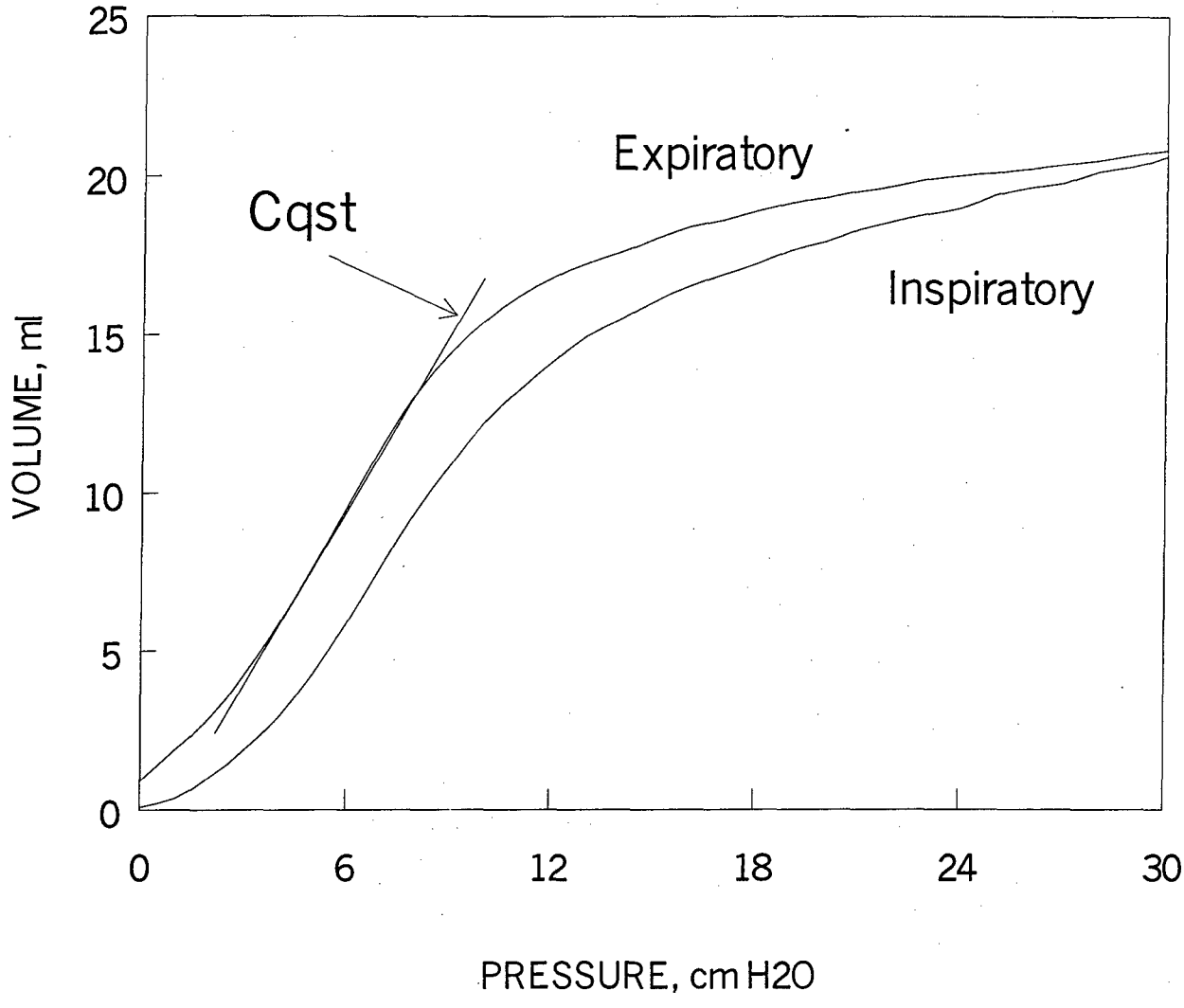
A picture of a Diamond Box (model PLY3114, Buxco Electronics, Sharon, CT) and control unit (Max II Maneuver Signal Generator, Buxco Electronics, Sharon, CT).

Figure 4. Single Breath Nitrogen Test



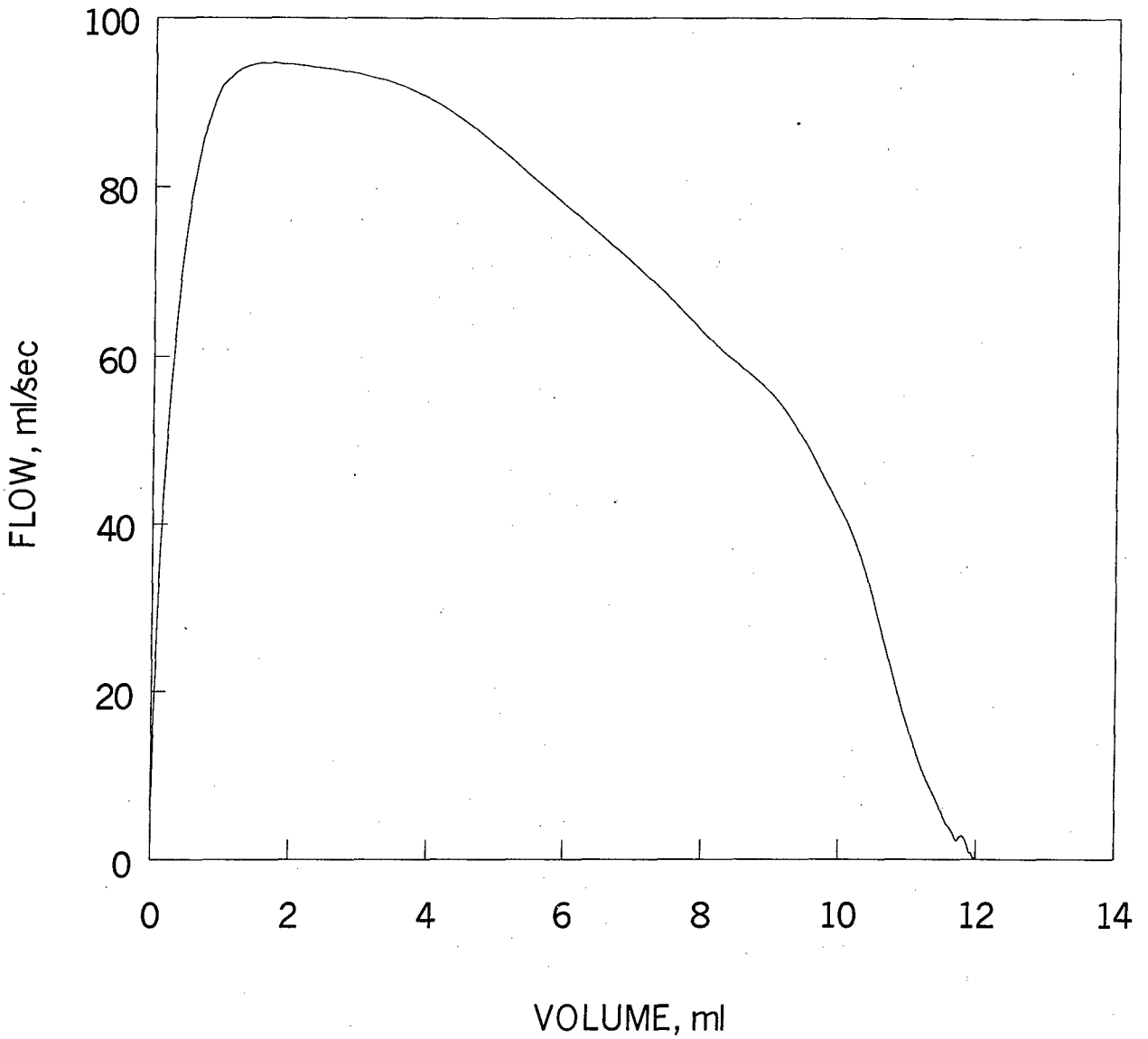
Single breath nitrogen test of a normal rat using a Diamond box. Airway nitrogen concentration of a rat measured versus volume change following a single breath of 100% oxygen. The slope of phase III was N_2 gas being washed out of alveolar units.

Figure 5. Pressure-Volume Curve



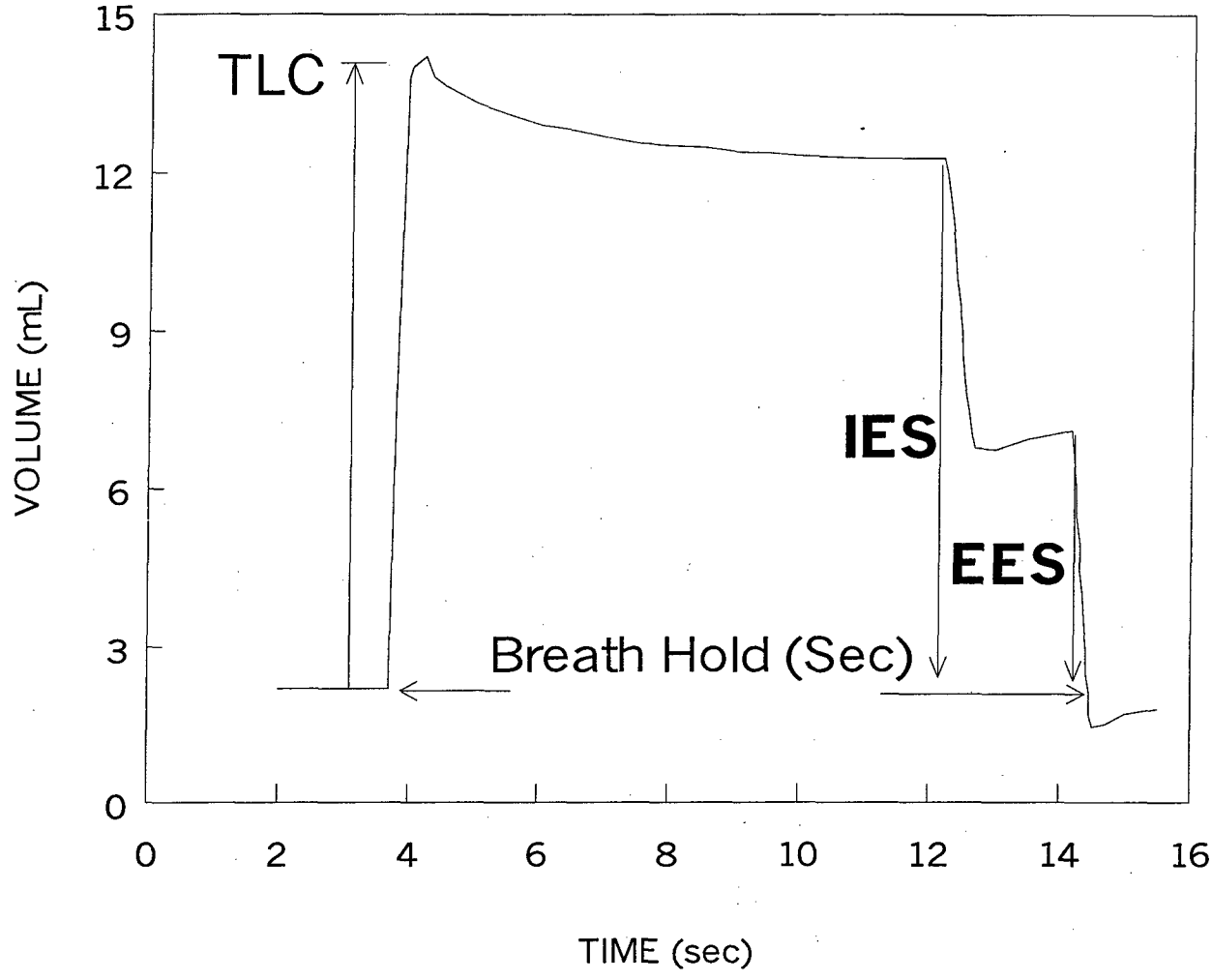
Pressure-volume relationships of the lungs and chest wall of a normal rat using a Diamond box. Airway pressure, measured with a pressure transducer, was plotted versus lung volume. Quasistatic compliance (Cqst) is the slope of the downward expiratory curve.

Figure 6. Expiratory Flow Volume Curve



Maximal expiratory flow volume curve measured from a normal rat during ventilation. The curve was generated by measuring flow in a Diamond box with sensitive transducers.

Figure 7. Diffusing Capacity (D_{LCO})



Schematic illustration of measured breath-hold time for a typical single-breath DLCO. Our method measured breath hold time from the beginning of inspiration to the end of alveolar gas sample collection. TLC = Total Lung Capacity, IES = Initial Expiratory Sample, and EES = End-Expiratory Sample.

Table 1. Ventilation and Respiration in Normal Rats

	Barometric Plethysmograph	*Volume Displacement w/ esophageal cannula*	*Diamond Box Plethysmograph w/ esophageal cannula*
Number of rats	9	12	30
Weights	223.0 ± 12.8	226.8 ± 12.9	271.0 ± 20.2
Ti	0.23 ± 0.05	0.30 ± 0.03	0.25 ± 0.04
Te	0.30 ± 0.07	0.40 ± 0.08	0.34 ± 0.18
TV	1.15 ± 0.24	1.47 ± 0.25	1.87 ± 0.27
f	121.4 ± 41.7	88.39 ± 11.2	113.8 ± 30.53
MV	130.7 ± 23.9	129.4 ± 25.2	206.8 ± 50.7
RT	0.19 ± 0.04	0.26 ± 0.09	0.11 ± 0.04
EIP	22.0 ± 4.3	14.7 ± 5.99	7.14 ± 1.24
PENH	0.49 ± 1.16	0.58 ± 0.40	3.02 ± 2.01
RL		0.33 ± 0.13	0.18 ± 0.05
Cdyn		0.60 ± 0.46	0.39 ± 0.11

Abbreviations and units: See figure 1.

Table 2. Ventilation and Respiratory Mechanics in Normal Rats

Reference	*Measurement (Mean ± Standard Deviation)*					
	Weight	f	TV	MV	Cdyn	RL
This Laboratory	223.0	121.4	1.15	130.7		
(1) Barometric	± 12.8	± 41.7	± 0.24	± 23.9		
(2) Flow Box	237.9	86.2	1.64	140.5	0.62	0.29
	± 9.1	± 20.3	± 0.22	± 40.5	± 0.35	± 0.09
(3) Diamond Box	271.0	113.8	1.87	206.8	0.39	0.18
	± 20.2	± 30.5	± 0.27	± 50.7	± 0.12	± 0.05
Palecek, 1969	229	112	1.8	203	0.44	0.59
	± 3.6	± 8.7	± 0.14	± 14.5	± 0.06	± 0.08
Palecek, 1969	220	104	1.3	131	0.37	0.50
	± 3.1	± 11	± 0.1	± 19	± 0.04	± 0.12
Robertson et al., 1965	318	69	0.93	64.2		
	± 16	± 4.7	± 0.04			
Diamond et al., 1977	233	115	1.4	161	0.25	0.26
	± 32	± 21	± 0.25		± 0.08	± 0.07
Thomas et al., 1969		139	1.11	150		
Chvalova et al., 1974	323	92.8	1.36	127	0.64	0.18
	± 33	± 5.7	± 0.05	± 8.3	± 0.05	± 0.01
Vizek et al., 1975	280	90	1.25	108		
	± 5	± 9	± 0.12	± 8		

Abbreviations and units: See Figure 1.

Table 3. Lung Volumes and Capacities on Normal Lungs

		This Laboratory (n = 30)		Harkema et al., 1982 (n = 16)	
	Units	mean	SE	mean	SE
Body weight	g	270.9	4.5	385	7
Total lung capacity (TLC)	ml	12.4	0.3	15.8	0.4
Vital Capacity (VC)	ml	10.1	0.2	14.1	0.3
VC/TLC	%	81	1	90	1
Functional Residual capacity (FRC)	ml	4.0	0.1	2.9	0.1
FRC/TLC	%	32	1	19	1
Residual Volume (RV)	ml	2.3	0.1	1.7	0.1
RV/TLC	%	18	1	10	1
Dynamic lung compliance (Cdyn)	ml/cm H2O	0.39	0.02	0.5	0.03
Slope III of single breath N2 washout	% N2/ml	0.4	0.07	0.48	0.03
Forced vital capacity (FVC)	ml	10.1	0.26	14.8	0.3
CO diffusing capacity (Dlco)	ml/min/mm Hg	0.16	0.003	0.3	0.01

Values of sub-divisions of lung volume. These values were collected from FRC determinations, PV curves, forced expiratory flow volume curves, and D_{LCO}.

Table 3. Continued.

		This Laboratory (n = 30)		Harkema et al., 1982 (n = 16)	
	Units	mean	SE	mean	SE
Forced vital capacity (FVC)	ml	10.4	0.28	14.8	0.3
Forced expired volume in 0.1 sec	% FVC	73	1.6	63	2
Forced expired volume in 0.2 sec	% FVC	96	1.1		
Forced expired volume in 0.4 sec	% FVC	100	1		
Mean midexpiratory flow (MMEF)	ml/sec	79	2.3	84	5
MMEF/FVC	FVC/sec	7.6	0.28	5.7	0.3
Expiratory flow at 50% (EF50)	ml/sec	84.3	2.5	90	5
EF50/FVC	FVC/sec	8.1	0.1	6	0.4
Expiratory flow at 25% (EF25)	ml/sec	52.9	1.9	50	3
EF25/FVC	FVC/sec	5.1	0.15	3.4	0.2
Expiratory flow at 10% (EF10)	ml/sec	22.5	1	17	1
EF10/FVC	FVC/sec	2.2	0.28	1.2	0.06

Values of sub-divisions of lung volume. These values were collected from FRC determinations, PV curves, forced expiratory flow volume curves, and D_{LCO}.

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13. **ABSTRACT** *(Maximum 200 words)* Pulmonary function tests (pfts) often are used in both humans and small animal species as physiologic biomarkers of pulmonary disease caused by the inhalation of toxic atmospheres. Physiologic biomarkers, used in conjunction with histopathological and biochemical biomarkers, can be used to diagnose disease, characterize dose/response relationships, assess disease pathogenesis, and indicate the degree of debilitation following pulmonary insult. We have developed methods to perform a battery of pfts that will be used to assess acute and chronic pulmonary toxicity in small animals exposed to inhaled toxins. The pfts developed can be used to assess physiologic responses in real-time during exposure, post exposure progress of induced pulmonary lesions, or both. Included are measures of ventilation (frequency, tidal volume, and minute ventilation), breath waveform analysis (flow derived parameters), dynamic pulmonary mechanics (compliance and resistance), static pulmonary mechanics (lung pressure-volume relationships and quasistatic compliance), sub-divisions of lung volume, pulmonary dynamics (forced maneuvers), distribution of ventilation (single breath N_2 washout), gas exchange (carbon monoxide - single breath diffusing capacity, microcapnometry), and measurement of metabolic activity (microcapnometry). Sixty one untreated Long Evans rats were used to develop the assays and to form a historical database for normal animals. Values for a variety of indices of pulmonary function measured using the methods developed in our laboratory were comparable to those reported by several other investigators.			
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